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Assessing the Impact of Pomegranate Juice on Liver Tissue in Male Rats Exposed to a Commercial Energy Drink

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The current study was designed to investigate the effect of pomegranate juice on some biochemical and histological variables resulting from dosing male rats with the energy drink Red Bull. In this study, 28 white male rats were used, divided into four groups, each involving seven animals, according to the following: G1 was given a physiological solution (5 ml/kg) and a standard diet for 120 days; G2 was given a dose of the energy drink Red Bull (volume 1 ml per 100 g); G3 was given pomegranate juice (5 ml/kg) first; then Red Bull; and G4 was given Red Bull first; and after six weeks, he was given pomegranate juice. The results showed that Red Bull consumption significantly elevated RBS, AST, ALT, TC, and TG levels. Pomegranate juice has the ability to reduce the concentration of RBS, AST, ALT, TC, and TG. Histological changes in the liver of animals that were given the Red Bull energy drink were represented by congestion of the central vein, degeneration, and necrosis of liver cells, with severe infiltration of inflammatory cells and a thick wall of the central vein (TW), as well as amyloid (AM) deposition, compared to the negative control group. The results of the histological examination showed that pomegranate juice could repair cellular tissue in the liver and make it similar to the tissue of a control normal group, denoting the possibility of using it in the diet and in treating atherosclerosis and heart disease.
Keywords: Pomegranate juice, liver, rats, energy drink, rat model, atherosclerosis, heart disease.

INTRODUCTION

Nutrients and their metabolites are construction blocks of cellular structures and energy sources. They also act as direct regulators of protein function and cellular signaling molecules and inducers or inhibitors of gene expression by directly modifying the activities of tissue factors or by changing epigenetic marks in the genome (Chen *et al.*, 2018), and thus, long-term consumption of unbalanced foods leads to cellular metabolic changes, which ultimately affect organismal metabolism (Zhu and Thompson, 2019). Energy drinks, including Red Bull, usually contain caffeine, with or without added nutritional supplements such as taurine, carbohydrates glucuronolactone, vitamins, and herbal ingredients such as ginseng, guarana, barbamati, cocoa, and kola nuts, which may increase the caffeine content without the consumer's knowledge. They are widely used by young people while studying, exercising, driving long distances, and during night outs (Bailey *et al.*, 2014; Curran and Marczinski, 2017) because they increase stimulation, concentration, mood, and activity. However, consuming energy drinks can increase the likelihood of developing metabolic syndrome. (Souza *et al.*, 2017). The plant kingdom is a significant source of potential medicines yet to be discovered. The use of herbal medicines for therapeutic purposes has become increasingly important due to the adverse effects and high toxicity of chemical-based medicines, which has led to a rationalization of their use in recent times. The pomegranate fruit, Punica granatum L., having high concentrations of ellagic acid, has received the attention of many researchers who have found many medical benefits in it (Maphetu *et al.*, 2022; Puneeth and Chandra, 2020). Studies proved that ligic acid has an antioxidant effect and suppresses the free radicals activity in cells, in addition to its antimutagenicity effect and its importance in reducing blood cholesterol, lowering blood glucose, and inhibiting the growth of many bacteria, fungi, and parasites. Studies have shown that energy drinks can have adverse effects on liver function, including elevated liver enzymes and oxidative stress, potentially leading to liver damage (Seifert *et al.*, 2011; McCusker *et al.*, 2006). These drinks often contain high levels of sugar and caffeine, which can contribute to liver inflammation and steatosis (Branco *et al.*, 2020).

In contrast, pomegranate juice, rich in polyphenols like ellagic acid and punicalagins, has demonstrated antioxidant

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and anti-inflammatory properties that can protect against liver damage in various models (Al-Malki and El Rabey, 2015; El-Beshbishy *et al.*, 2010). However, there is limited research specifically examining its potential to mitigate the negative effects of energy drinks on the liver.

This study hypothesized that consumption of pomegranate juice will mitigate the adverse effects on liver tissue caused by exposure to a Red Bull energy drink in male rats.

Our current study aimed to investigate the effect of pomegranate juice on some histological and biochemical variables resulting from dosing male white rats with the Red Bull energy drink.

MATERIALS AND METHODS

***Materials*:** This study used 28 male white Swiss rats (Sprague Dawley), aged 3-4 months old and weighing between 170-210 g. They were placed in plastic cages measuring 15x20x30 cm with metal covers prepared for this purpose in the animal house of the Department of Biology/ College of Education for Pure Sciences / University of Anbar. The animals were subjected to laboratory conditions of a light period divided into 11 hours of light and 13 hours of darkness. The temperature was fixed at 22±2 C. Care was taken to clean the cages and sterilize them every week, where they left for two weeks to adapt to the new conditions and ensure they were free of diseases. Beside, the experimental protocol was approved by a Scientific Research Ethics Committee at ministry of health to maintain ethical guidelines for research investigations, with (Approval No; 27325/2019).

***Material preparation*:** Red Bull energy drink was obtained from the local market as a 250-ml can containing about 27 g sugar (glucose, sucrose), 100 mg taurine, 600 mg glucuronolactone, vitamin B, and 80 mg caffeine (RED BULL ENERGY).

***Pomegranate Juice preparation*:** Pomegranate fruit obtained from local markets was used. It was cleaned and peeled, the fruits were squeezed entirely, and the resulting juice was filtered and used for dosing according to (Al-Moraie *et al.*, 2013).

***Experiment Design*:** The experiment was conducted for the period between the beginning of January and the end of March. The animals were divided according to similar weights into four groups, each group involved 7 animals, as shown below:

- First group (G1) (control): They were dosed orally with 5ml/kg of distilled water.
- Second group (G2) (Red bull): They were dosed orally with energy drink (Red bull) at 0.1 ml/g daily.
- Third group (G3) (Preventive): Rats were dosed first with pomegranate juice at ml/kg, and two hours later, they were given the energy drink (Red Bull) at the same volume.
- Fourth group (G4) (Therapeutic): The rats were dosed with the energy drink (Red Bull) first at the same volume for six weeks, then the dosage was stopped for one day, and after that, they were given only pomegranate juice at the same quantity above for six weeks, too.

The experiment lasted 120 days, and the dosing was every other day.

***Blood sampling*:** After starving the animals for 24 hours, they were anesthetized using chloroform, and blood was drawn directly from the heart with a sterile disposable syringe (5 mL), placed in white tubes, and kept at room temperature until coagulated. Next, it was centrifuged at a speed of 3000 rpm for 15 minutes. Then, the serum was separated with a micropipette and stored in plastic test tubes at a temperature of -20°C, recording all information related to each sample until biochemical examinations implemented the procedure.

***Biochemical variables*:** The following variables were recorded according to the method mentioned next to each of them.

1. Serum Glucose: The glucose level in the blood serum was measured with a glucose test kit imported from the Spanish company Linear, according to the method of Dingeon *et al.* (1975).
2. The percentage of ALT and AST in the blood serum was measured using a kit provided by the Spanish company Linear, relying on the ultraviolet enzymatic measurement method with a spectrophotometer (Tietz, 1995).
3. The triglyceride concentration in blood serum was estimated using the analysis kit (Kit) provided by the Spanish company (Linear), according to the method referred by Fossati and Prencipe (1982).
4. The cholesterol concentration in the blood serum was measured using the analysis kit provided by the Spanish company (Linear), according to the method of Allain *et al.* (1974).

Histological variables: Histopathological changes in liver tissues of all experimental animals were evaluated. The rats were dissected, and then the liver was taken and preserved in 10% formalin to fix the tissues. They were then passed through ascending stages in an ethanol solution, thinned with xylene, and Imbibited and embedded in paraffin cubes. After that, thin sections (5–7 µm) were stained with hematoxylin and eosin (H&E) solution and examined under a compound light microscope equipped with a digital camera.

***Statistical analysis*:** Data were statistically analyzed with software Package for Social Science (SPSS) calculate the least significant difference between the treatments according to Duncan' test.

RESULTS

***Biochemical analysis*:** Results of the current study (Fig. 1, 2, and 3) showed a significant increase in the level of glucose concentration, total cholesterol (TC), and triglycerides (TG)

at the probability level (P≤0.05) in the blood serum of male rats that were dosed with Red Bull energy drink (G 2).

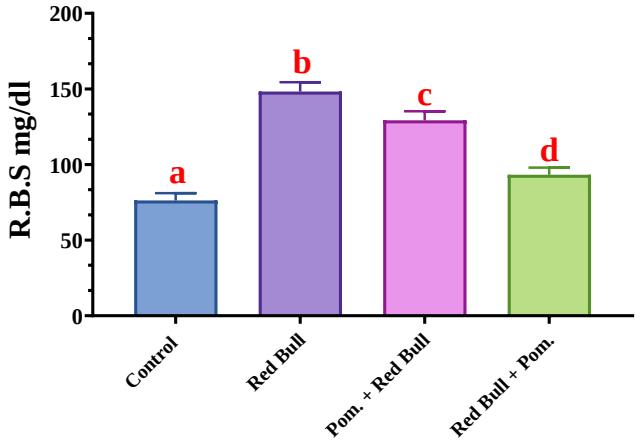

Figure 1. Effect of pomegranate juice on the concentration of glucose (RBS) in male rats treated with the energy drink Red Bull.

- The number of animals is seven for each group.
- Different letters indicate the presence of significant differences (P≥0.05) within the 120-day experimental period

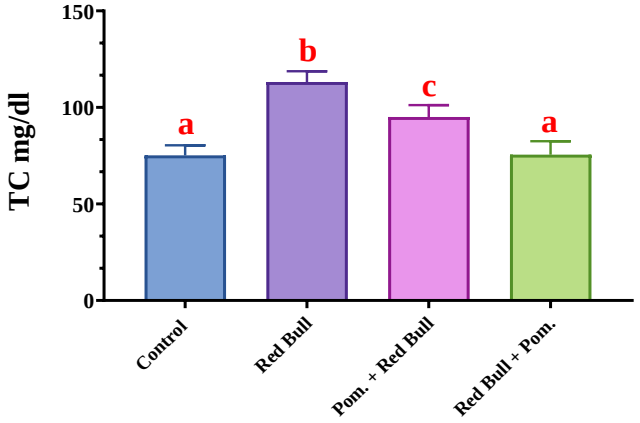

Figure 2. Effect of pomegranate juice on the total cholesterol concentration in male rats treated with the energy drink Red Bull.

- The number of animals is seven for each group.
- Different letters indicate the presence of significant differences (P≥0.05) within the 120-day experimental period

The results also showed a significant decrease in the concentration of glucose, total cholesterol (TC), and glycerides in the blood serum of male rats dosed with Red Bull first and then with pomegranate juice (the fourth group). At the same time, there was an insignificant decrease in the third group (G3), dosed with pomegranate juice first, then Red Bull drink, compared to the second group.

Results of the current study, displayed in Fig. (4 and 5), showed that there was a significant increase at the probability level P≤0.05 in the activity of the enzymes AST and ALT in the blood serum of male rats that were dosed with Red Bull at compared to the natural control animals, while the activity of the enzymes AST and ALT in the blood serum significantly decrease at the probability level P≤0.05 in of male rats of the third group (preventive) that were given pomegranate juice first and then Red Bull drink.

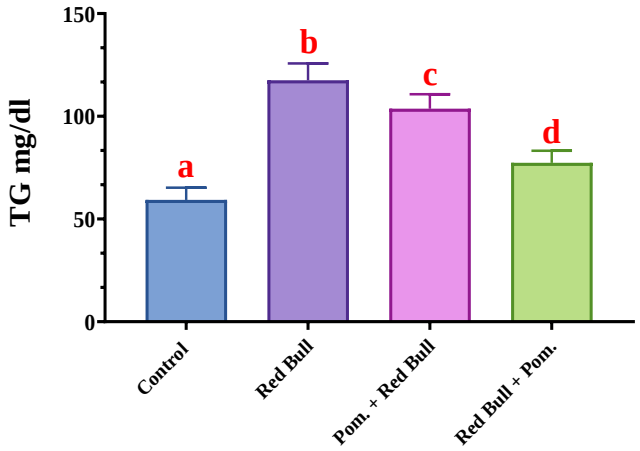

Figure 3. Effect of pomegranate juice on the triglycerides (TG) concentration in male rats treated with the energy drink Red Bull.

- The number of animals is seven for each group.
- Different letters indicate the presence of significant differences (P≥0.05) within the 120-day experimental period.

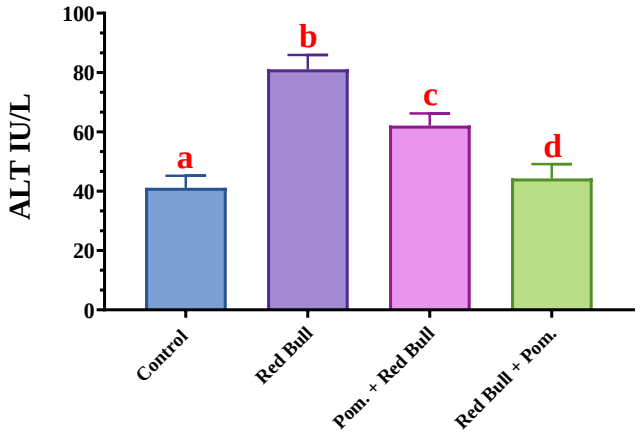

Figure 4. Effect of pomegranate juice on the AST enzyme activity in male rats treated with the energy drink Red Bull.

- The number of animals is seven for each group.
- Different letters indicate the presence of significant differences (P≥0.05) within the 120-day experimental period.

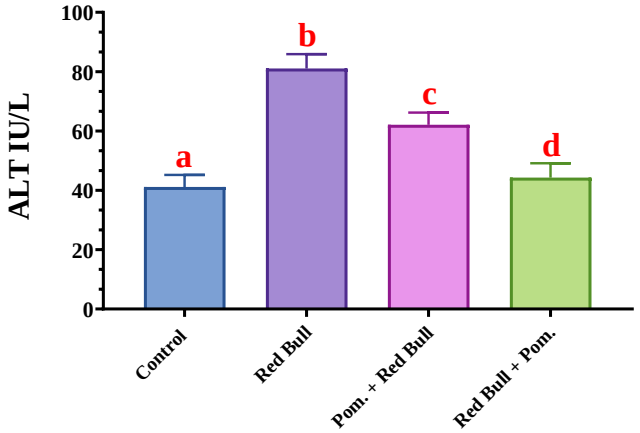

Figure 5. Effect of pomegranate juice on the ALT enzyme activity in male rats treated with the energy drink Red Bull.

- The number of animals is seven for each group.
- Different letters indicate the presence of significant differences (P≥0.05) within the 120-day experimental period.

Histological changes in the liver: Microscopic examination results of liver tissue sampled from the groups of control male rats showed a normal histological pattern, as it contains lobes of hepatocytes that appeared normal and arranged in a radial pattern towards the center of the lobule containing the central vein. These cells are polyhedral in shape, have an acidic eosinophilic cytoplasm, and have spherical nuclei. More than one nucleus can be distinguished in some hepatocytes, interspersed with well-formed sinusoids, in which small numbers of Kupffer cells are observed. The portals have branches of the hepatic artery, hepatic portal vein, and bile duct branch (Fig.6)

The histological study results of the liver in the group of rats that were dosed with Red Bull energy drink (G2) showed severe histological changes, including thickness in the wall (TW) of the central vein, degeneration (D), and necrosis (N) of some hepatocytes. In addition to the presence of blood congestion (CON), as well as the presence of lymphocyte infiltration (LI) and the deposition of amyloid (AM) (Fig.7 and 8). Microscopic examination of studying through the liver histological sections of the rat group that first drank pomegranate juice and then the energy drink Red Bull showed the occurrence of histological changes, including the presence of thickness in the wall (TW) of the central vein with a state of blood congestion (CON), as well as infiltration of some lymphocytes (LI), and the histological study results showed degeneration (D) of some liver cells (Fig.9 and 10), while the results of the histological examination in the group that was dosed first with Red Bull and then pomegranate juice showed the appearance of a normal pattern of liver tissue similar to the control group (Fig. 11). Moreover, the histological results showed an improvement in the liver cells in the fourth group after giving pomegranate juice.

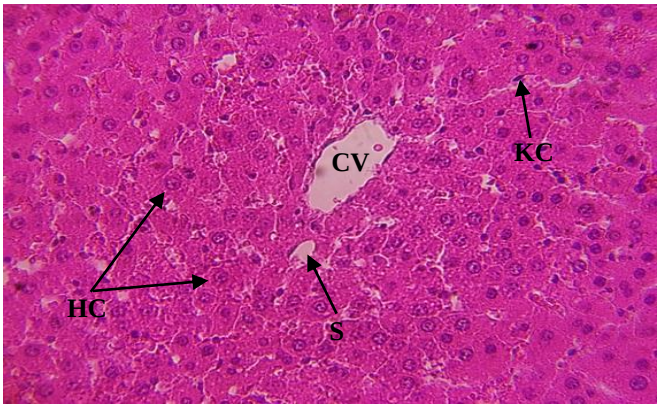

Figure 6. A section of the control group liver illustrating the normal appearance of the central vein (CV), hepatocytes (HC), and sinusoids (S) with H&E X400 KC cells

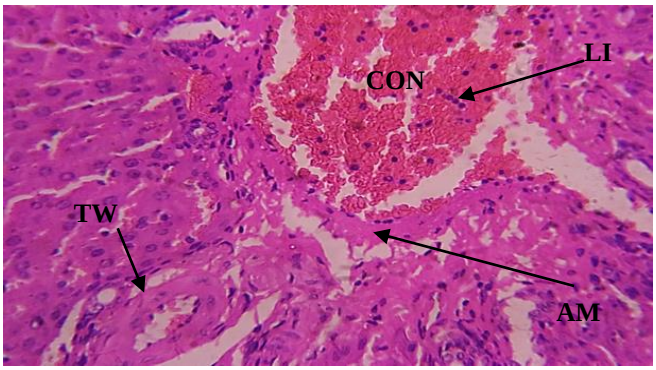

Figure 7. A section of the Red Bull group liver illustrating the thick wall (TW) of the central vein, blood congestion (CON), and amyloid deposition (AM) with lymphocytic infiltration (LI) H&E X400.

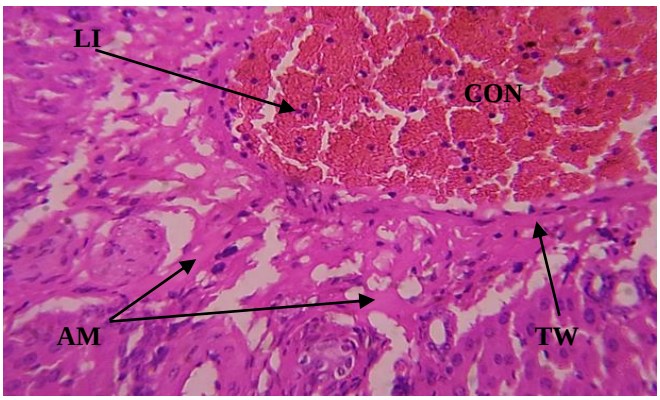

Figure 8. A section of the Red Bull group liver illustrating the thick wall (TW) of the central vein, blood congestion (CON), and amyloid deposition (AM) with lymphocytic infiltration (LI) H&E X400.

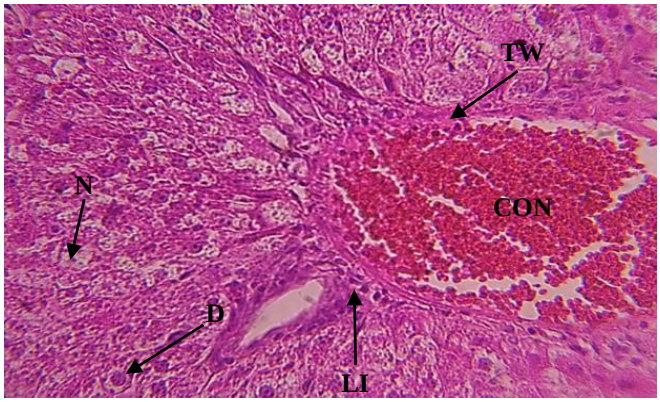

Figure 9. A section of the liver of the pomegranate group and then the red bull group showing the thick wall (TW) of the central vein, degeneration (D) of hepatocytes, and congestion of blood (CON) with lymphocytic infiltration (LI) H&E X400.

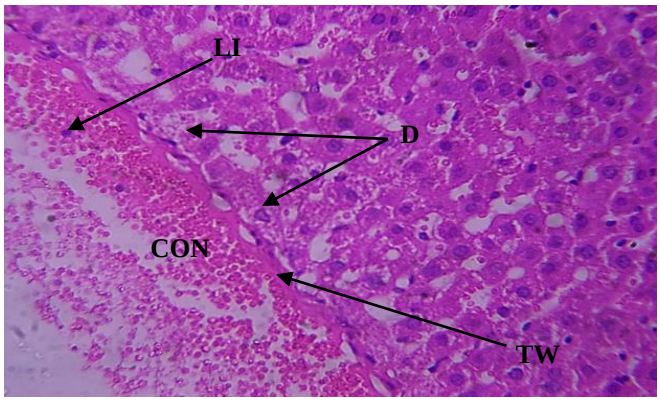

Figure 10. A section of the liver of the pomegranate group and then the red bull group showing the thick wall (TW) of the central vein, degeneration (D) of hepatocytes, and congestion of blood (CON) with lymphocytic infiltration (LI) H&E X400.

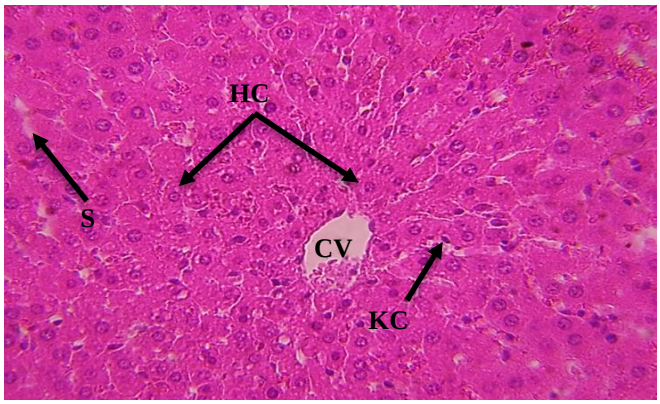

Figure 11. Section of the liver of the Red bull and pomegranate group showing the central vein (CV), hepatocytes (HC), and sinusoids (S) with KC cells, H&E X400.

DISCUSSION

The hyperglycemia level in the group that was dosed with Red Bull drink may be due to the increased generation of free radicals resulting from giving the energy drink in high quantities, which contributed to the destruction of pancreatic beta cells and inhibited their work, as the free radicals stimulate the process of lipid peroxidation and inhibit the insulin hormone production, thus leading to inhibiting the insulin secretion and increasing in glucose concentration (Bukhar *et al.*, 2012).

Increased consumption of energy drinks has harmful effects, including increased white adipose tissue, inflammation, and enhanced insulin resistance, all of which are linked to caffeine, taurine, and sugar (El Desouky *et al.*, 2019). Also, regarding the effect of taurine, a study on rats demonstrated that taurine supplements enhance insulin resistance caused by high-fat content and reduce insulin secretion by pancreatic cells, thus increasing blood glucose (Eltahir *et al.*, 2020).

The results referred to a significant decrease in blood glucose concentration in the third group (pomegranate juice first, then the energy drink) and the fourth group (the energy drink first, then pomegranate juice), which perhaps due to the active components in pomegranate juice, including the alkaloids Pelletierine, which is also called Punicine, Pseudopelletierine, Isopelletierine, Methyl Isopelletierine, Granatin, Tannin, Resine, Sugars and Starch. It also contains malic acid and citric acid that may increase the glucose transfer across the cell membranes and thus increase its utilization by the cells or analyzing to produce energy (Taheri Rouhi *et al.*, 2017). It should also be noted in this domain that the reducing action of pomegranate juice may come through different mechanisms, as its active sugar-linked components, such as Ellagic acid and flavonoids, are of four types, namely Kaempferol, Quercetin, Naringenin, and Luteolin. These biologically active compounds may reduce the glucose absorption rate by the intestine and stimulate insulin secretion by pancreatic beta cells (Virgen-Carrillo *et al.*, 2020), as well as possibly stimulate the peripheral use of sugar by adipose, muscle, and nervous tissues, directly or indirectly, by increasing sensitivity to insulin with a synchronous decrease in the glucose synthesis process.

The increase in both cholesterol and triglycerides in the serum of male rats that were dosed with the Red Bull energy drink, illustrated in the table, may be due to several reasons, including an increase in the activity of the enzyme Cholesterol Acyl Transferase, which is responsible for the cholesterol absorption. This enzyme, in turn, is stimulated at insulin lack due to oxidative stress, resulting from high concentrations of free radicals, because of the components found in energy drinks, which reduce the activity of the lipoprotein lipase enzyme present in the body's tissue, which is responsible for decomposing triglycerides into fatty acids and glycerol (Famurewa *et al.*, 2015); or this increase in cholesterol may

be because continuous treatment with an energy drink, affecting the composition of the intestinal monolayer structure and increasing the cholesterol absorption through the intestine. The oxidative stress resulting from treatment with Red Bull also decreases insulin secretion due to its effect on the pancreatic β-cells and the destruction of part of them, leading to a decline in the effectiveness of the lipoprotein lipase enzyme, which is responsible for decomposing triglycerides into fatty acids and glycerol (Muxiddinovna, 2022). Moreover, due to stress and decreased insulin, fat decomposition in adipose tissue is stimulated, and free fatty acids (FFA) are released as energy sources. Furthermore, it also participates in constructing TG. In addition, the liver takes large amounts of FFA to increase its production of VLDL, which increases the TG concentration in the blood serum. (Eltahir *et al.*, 2020).

The results above also exhibited a significant decrease in the total cholesterol concentration in both the third and fourth groups that were given pomegranate juice before and after the energy drink compared to the second group, which may be due to the pomegranate juice content of active compounds. The most important of these compounds is Peletierteing, which inhibits emptying and enhances the process of excreting fats from the digestive tract (El-Sayyad *et al.*, 2020). Pomegranate also contains alkaloids, glycosides, terpenes, and saponins, which may lead to the inhibition of the hepatic enzyme Hydroxy methylglutaryl reductase CoA involved in cholesterol synthesis, thus reducing the concentration of cholesterol and the intermediates involved in its synthesis (Binmowyna *et al.*, 2020). Compounds in pomegranate juice also have the ability to remove free radicals ($OH\cdot$, $^1O_2$, and $O_2^{-}\cdot$) from the body, thus reducing the oxidation of LDL-C and lowering cholesterol (Taheri Rouhi *et al.*,2017), which may be due to the presence of high concentrations of monounsaturated fatty acids (MUFA), which prevent the oxidation of LDL-C and reduce the concentration of cholesterol in blood serum. So, pomegranate juice is characterized by its antioxidant action, removing free radicals (ROS, RNS) and inhibiting LDL-C oxidation. C and decreased cholesterol concentration. These compounds may also inhibit cholesterol absorption from food in the intestine, inhibit its liver production, stimulate bile secretion, and excrete it with fecal waste outside the body (Ramzy, 2019). The reason may also be that pomegranates contain sugars, proteins, fatty substances, and organic acids, including citric and malic acid. Pomegranate peels contain 28% tannin, and the fruits contain 10% sugars, 1% citric acid, 84.2% water, 20.1% fiber, and small amounts of iron, phosphorus, sulfur, calcium, manganese, and potassium, with the presence of vitamins, including (A, B, E). In pomegranate seeds, the fatty substance reaches 9.7%, which can help to protect the pancreatic beta cells responsible for insulin secretion from damage. Increasing insulin levels has a vital role in activating the enzyme lipoprotein lipase in adipose tissue, which is responsible for degrading triglycerides (Hassan *et al.*, 2018). The imbalance in the food metabolism and an increase in the glucose level in the blood are often accompanied by changes in the activity of some enzymes, especially metabolic enzymes. Therefore, measuring the activity of these enzymes in blood serum is clinically beneficial and may help detect some tissues affected by an increase in the activity of an enzyme specific to a particular tissue. Therefore, liver enzymes such as AST and ALT were studied. The results of the current study referred to an increase in the levels of the two enzymes above in the blood serum of animals treated with Red Bull. This result was consistent with many studies on laboratory animals dosed with energy drinks. The increase in the ALT enzyme, primarily caused by the breakdown of liver cells, is usually accompanied by an increase in the AST enzyme, as the ALT enzyme is present in the liver at a rate three times greater than its presence in the heart and kidney muscle. An increase in the AST enzyme also indicates an increase in the ALT enzyme, denoting damage or destruction in hepatocytes, followed by destruction in cardiac tissue (Costa-Valle *et al.*, 2018). Therefore, the reason for the increase in the activity of the two enzymes may be due to the metabolic imbalance resulting from giving an energy drink, which increased the hepatic cell metabolism and thus increased the leakage of liver enzymes. The activity increase of these two enzymes may also be due to hepatocyte enlargement and the stimulation of the endoplasmic reticulum to produce a larger amount of the enzyme proportional to the cell size (Mansy *et al.*, 2017). Also, the increase in the fat percentage, especially cholesterol, in the liver creates what is known as liver steatosis, where the fatty acids accumulate due to the high glucose level in the blood, where the same enzymes increase as a result (Valle *et al.*, 2018). This is in addition to the role of oxidative stress and the formation of free radicals, resulting in the destruction and necrosis of liver cells, causing the leakage of enzymes into the bloodstream. These radicals also cause waxing and damage to the liver tissue and the loss of enzyme receptors located on the epithelial cells lining the bile duct and around the central vessel, causing an increase in releasing enzymes outside the cells. It may be due to dyslipidemia, as high cholesterol leads to high levels of liver enzymes, which reflects fatty liver disease (Gubina-Vakulyck *et al.*, 2020). Perhaps there is another reason why energy drinks may cause liver damage and an increase in the level of liver enzymes, that is liver cell poisoning, resulting from oxidative stress, which causes plasma membrane destruction and thus the destruction of most cell membranes due to the peroxidation of the fats that interact with the polyunsaturated fatty acids of the liver cell membrane, which eventually change the membrane permeability, and thus causing leakage of liver enzymes into the blood serum (Eltahir *et al.*, 2020). The decrease in the activity of the enzymes mentioned above after treatment with

pomegranate juice in the third and fourth groups compared to the second group of animals is clear, which may be due to the pomegranate containing antioxidants represented by alkaloids, glycosides, flavonoids, saponins, sterols, tannins, and amino acids such as glutamine, cysteine, and glycine, in addition to the presence of fatty acids, including α-linolenic acid and linoleic acid, which performance is like detergents in eliminating free radicals (Noori *et al.*, 2017). These results denote the antioxidant effectiveness of pomegranate juice against liver damage caused by excessive consumption of energy drinks, as it protects liver mitochondria and microsomes from membrane-destructive changes, especially lipid peroxidation, and thus prevents cell enzymes from being released into the blood (Hassanen *et al.*, 2019). The decrease in these enzymes may be because pomegranates contain fatty acids, including oleic acid, which is characterized by having an influential effect against free radicals, reducing their toxic effect on liver cells and improving their functions. Thus, enzymes act as an antioxidant and reduce oxidative damage to liver cells. We also believe that the decrease in the activity rate of the enzymes AST and ALT in the sera of animals treated with pomegranate juice may be due to its role in contributing to repairing cell membranes, thus maintaining normal enzyme concentrations within the liver cells or in raising the level of antioxidants (Pawankalyan *et al.*, 2022).

The liver is the main organ responsible for metabolism related to detoxification, through which the body gets rid of the most considerable possible amount of toxic substances by breaking down unwanted substances.

The histological changes that occurred in the second group were consistent with many studies that clarified the harm caused by excessive consumption of energy drinks, referring to the enlargement of liver cells as a result of fat accumulation in liver cells for the group of male rats subjected to the dosing with energy drink (Mukhiddinovna, 2022). This liver harm is because excessive consumption of Red Bull causes oxidative stress, including fat oxidation, and as a result, it stimulates the process of programmed death of liver cells and causes tissue apoptosis (Khayyat *et al.*, 2015), and the fat peroxidation occurring in the liver cell membrane, which stimulates a series of membrane endolytic reactions with a decrease in the vitality of the mitochondrial membrane, accumulation of MDA, and destruction of the membranes of lysosomes; hence the cell reaches the stage of necrosis (Al-Eryani *et al.*, 2018). Furthermore, energy drinks disrupt antioxidant defense mechanisms in tissues, especially GSH and CAT, by disrupting some enzyme systems in the liver, which is shown by our results through biochemical tests, thus causing a group of lesions and histological changes in the liver described above (Munawar *et al.*, 2016), as malfunctioned liver may lead to a change in the level of liver enzymes, represented by their increase. Researchers reported that the toxins resulting from (Red Bull) may cause a change in the cellular membrane permeability due to their effect on the membrane structure and fluidity, which results in an increase or decrease of material permeability and then the entry of substances that affect nucleic acids, or prevent the entry of energy sources and the exit of metabolic products, accompanied by free radicals whose reaction form complex compounds, affecting the cells and combine with their molecules, causing necrosis and cell death (Khayyat *et al.*, 2015).

Toxicity can be removed by glutathione, one of the protective defense means inside the cell. When the toxicity exceeds the ability of antioxidants, it binds to liver cells and causes necrosis. Also, excessive consumption of energy drinks reduces antioxidants and upsets the state of balance between oxidants and antioxidants (Ibrahem *et al.*, 2022). Necrosis is also due to the expansion of the central hepatic veins (blood vessels) and a change in their permeability due to the toxic effect and liver infections, which affect the access of oxygen and food to the liver cells, causing cell malfunctions or death. Damage to liver cells is accompanied by an increase in the concentration of the enzymes ALT and AST resulting from excessive consumption of Red Bull, affecting liver cells' goodness and increasing cellular leakage (Mahmood and Thanoon, 2021). Here, it is worth mentioning that the results of the malondialdehyde and glutathione test exhibited an increase in MDA, which is an indicator of fat peroxidation due to the formation of free radicals, making us able to conclude that the resulting degenerative effects in liver tissue are due to the formation of free radicals, an increase in MDA and a decrease in the level of GSH. The bleeding occurs in the tissue due to increased pressure within the vessels, causing a breakdown in the vessel wall and blood cell expulsion. When toxic substances react with glutathione, certain compounds cause blood cell decomposition. This can eventually lead to liver failure and venous congestion, which can cause the sinusoids and hepatic veins to expand, generating pressure on the hepatocytes. (Ali, 2019).

The necrosis occurrence is due to the difference in the distribution of enzymes in liver parts, the type of cells in terms of function, or hematological supply; during blood impact, the surrounding areas are harmed first (Munawar *et al.*, 2016). Caffeine affects the metabolism of carbohydrates and fats in the liver and causes oxidative stress. The presence of caffeine in quantities with simple sugars increases insulin resistance, and as a result, metabolic disorders develop in the liver. The niacin B3 metabolism leads to the release of nicotinamide and pyrimidine after a series of oxidation and reduction reactions. The first compound leads to a series of oxidation reactions resulting in oxidative stress by inhibiting beta-oxidation, which leads to a dysfunction in the mitochondria and, thus, a defect in the adenosine triphosphate (ATP) production, which leads to programmed cell death or necrosis of liver cells, which is the cause for releasing cytokines that lead to inflammation, including interleukin, tumor necrosis factor TNF-α, and interferon INF-g by Kupffer cells and natural killer cells, which results in inflammation and tissue damage

(Al-Eryani *et al.*, 2018). The results of this study explain that enzymes have leaked in high quantities from the liver tissue into the body fluids, especially the serum, which reflects the damage occurring in the body's tissues, specifically the liver, as it is the main organ in the body responsible for treating toxins to which the body exposes.

The histological results showed an improvement in the liver cells in the fourth group after giving pomegranate juice, which may be due to it containing active compounds, especially Punicalagin, Punicalin, and Ellagitannins, which have a significant role in therapeutic and preventive effects, since they remove free radicals, prevent fat oxidation, and protect hepatocytes against damage (Hassan *et al.*, 2018). These effective compounds are also able to reduce hepatic cell necrosis and inhibit and kill deformed, diseased, or senescent cells that suffer from severe cellular degeneration; moreover, the pomegranate juice has an ability to stimulate cellular antioxidant processes and increase metabolic processes and function of the liver, thus enhancing the cellular structure and function (Noori *et al.*, 2017). Hydrolyzable tannins, found in pomegranates, are potent antioxidants that can scavenge free radicals and protect the body from damage caused by them(Ali, 2017; Noori *et al.*, 2017); in addition to their role in reducing the body's cholesterol; furthermore, there are some essential compounds in pomegranates such as Quercetin, Kaempferol, ellagic acid, and Rutin which inhibits the lipid oxidation in hepatic cell membranes by removing free radicals (Faddladdeen and Ojaimi, 2019). Additionally, active components in pomegranates, including flavonoids, saponins, alkaloids, and some vitamins, especially vitamin C, have a role in seizing effective oxygen species and increasing the activity of SOD and CAT, by producing the energy necessary for their work, which has been exhausted as a result of oxidative stress by Red bull components. Therefore, ATP depletion is an effective indicator of cell damage and death(Rajab and Ali, 2020) , and these results reveal the ability of the active components of pomegranate juice to repair cellular liver cells affected by (Red Bull) or inhibit its toxic action (Helal Alzahrani and Mohammed Abd Elmeged, 2022). From this, we can denote the role of the pomegranate juice components, including phenols, saponins, glycosides, some vitamins, and others, in inhibiting the degenerative action through their possession of many activities, including antioxidants, their ability to seize the formed free radicals, and their ability to inhibit the agent causing inflammation, and then accelerate the process of cellular repair and stimulate anti-inflammatory cells, including macrophages. In addition, some components in pomegranate have the ability to stimulate the tissue to divide to compensate for damaged cells, and they also have the ability to inhibit the growth of cancer cells in liver tissue exposed to carcinogenic chemicals by stimulating the programmed death process of these cells (Pawankalyan *et al.*, 2022). Therefore, we can conclude that pomegranate juice significantly reduced fat peroxidation by returning antioxidants to their standard l level, and thus, the liver tissue became closer to its normal composition.

Pomegranate juice has the ability to protect the liver from Red Bull-induced damage in rats through several mechanisms including: antioxidant activity (Dhanya *et al.*, 2014) and anti-inflammatory effects (Amri *et al.*, 2012). Additionally, its ability to support detoxification processes and regulate lipid metabolism (Rasheed *et al.*, 2020) could contribute to liver cell protection against the harmful effects of energy drink (Red Bull).

Conclusion: The study suggests that pomegranate juice could protect and repair the liver from damage caused by factors like energy drinks. However, more research is necessary to fully grasp and utilize the therapeutic advantages of pomegranate juice for liver health. Future efforts should prioritize human trials to confirm its effectiveness and safety, test different ways to use it, study its long-term effects on liver diseases, understand how it works on a molecular level, check for interactions with medications, and explore how it affects various populations' liver health. These steps will help us better understand and leverage the potential benefits of pomegranate juice for liver-related conditions.

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